

ABSTRACT

The invention refers to mono- and divalent (diabody) single chain Fv (scFv) antibody fragments, obtained by recombinant DNA techniques from the anti-carcinoembryonic antigen (CEA) monoclonal antibody (Mab) CB/ior-CEA.1. This antibody has high affinity for CEA 5 and is employed in the diagnosis and follow-up of human colorectal tumors. As the original Mab, the monovalent fragment and the diabody exhibit high affinity for human CEA and recognize an epitope dependent of carbohydrate conservation. The monovalent scFv fragment and the diabody have affinity constants for CEA of $(5.0 \pm 0.4) \times 10^9 \text{ L mol}^{-1}$ and $(2.8 \pm 0.3) \times 10^{10} \text{ L mol}^{-1}$, respectively. These two fragments do not show cross reactivity with human 10 normal cells and tissues, exception made of the normal colonic mucosa, where CEA is occasionally present. The fragments can be produced through the expression in recombinant microorganisms, starting from the cloning of the encoding variable region nucleic acid sequences obtained from the hybridoma that produces Mab CB/ior-CEA.1. As the original Mab, the monovalent scFv and the diabody have the ability to identify *in vivo* cells that 15 produce human CEA and grow as tumors in mice. The monovalent scFv and the diabody have a molecular size 5 and 2.5 times lower, respectively, than the mouse Mab, and do not have Fc domains, fact this that confers them the potential to better penetrate tissues *in vivo* and to be less immunogenic in man.